GENERATIONAL "CARRYOVER" AND THE SUPPRESSION OF SUBMARGINAL PATTERN ELEMENTS IN VERNAL PHENOTYPES OF *PIERIS PROTODICE* (LEPIDOPTERA: PIERIDAE)[1]

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The Checkered White butterfly, *Pieris protodice* Boisduval and LeConte, like many Pieridae exhibits a photoperiodically controlled seasonal polyphenism (Shapiro, 1968). The vernal-autumnal phenotype (*"vernalis"* Edwards), produced from larvae reared under September and October photoperiods, is characterized by heavy dark scaling on the veins of the hindwing beneath and by a series of chevron-shaped markings between these veins (figure 1a). This pattern is characteristic of the species-group to which *P. protodice* belongs, although it is reduced in summer phenotypes of multivoltine species and in both generations of the partially bivoltine *P. sisymbrii* Boisduval. In this species the chevron-markings, while never complete, are sufficiently recognizable to present a facies easily distinguishable from that of the *P. napi* species-group, in which only the veins are melanized. Specimens of *P. protodice "vernalis"* resembling *P. sisymbrii* in this character are occasionally taken wild, and very rarely individuals may occur in which the chevrons are completely suppressed. Examination of long bred and wild series of *P. protodice* reveals that the chevrons are composed of lines extending basad from the vein-tips and converging near the middle of the interspace; they frequently fail to meet and form a vertex. When the vein-lines are unusually heavy they may absorb the chevron-lines to the extent that the pattern is obscured (as in *P. sisymbrii)* or effectively obliterated (as in the extreme *P. protodice* figured by Shapiro, 1969). The bred male shown in figure 1b displays the usual manifestations of "chevronlessness."

Prior to 1971 no "chevronless" *P. protodice* were produced in laboratory rearing. In July, 1971 a stock was established from

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three females collected by Richard Pine in Brooklyn, Kings Co., New York. After one generation on a long-day regime (14 hours light: 10 dark, 25°C), part of this stock was carried through five consecutive generations on a short-day, high-temperature regime (8 light: 16 dark, 25°C both phases) which induces *"vernalis"* but inhibits diapause. Numbers of *"vernalis"* were desired for field survivorship studies; breeding in each generation was from five females each mated once to a different male, and allowed to oviposit for two days. No "chevronless" butterflies were seen in the first generation, but in the second 26 of 110 butterflies showed significant suppression of the marginal chevrons. Although "chevronless" butterflies were not used for breeding, the character continued to appear at a nearly constant frequency through the fourth generation and then dropped in the fifth (table 1). The line had to be discontinued at this time for reasons unrelated to the experiment.

Two third-generation "chevronless" butterflies were paired, as were two normal *"vernalis,"* and the females were allowed to oviposit until they died (four and six days, respectively) to maximize the number of progeny. Rearing was on the same regime. The resulting phenotypic distributions (table 2) do not differ significantly from each other or from the corresponding (fourth) generation in the main culture. When rearing is in a uniform environment, individual variation should reflect genetic differences in the developmental responses of the individuals to that environment. This very limited rearing does not, however, indicate any simple genetic basis for "chevronlessness."

The significance of the variation in the frequency of "chevronlessness" over five generations of uniform rearing is also obscure. Interestingly, it was coupled with variation in the frequency of light and intermediate (i.e., non-*"vernalis")* phenotypes (table 1). While "chevronless" was holding essentially constant, non-*"vernalis"* phenotypes increased greatly in frequency in the third and fourth generations, but dropped equally dramatically in the fifth. The fifth generation phenotypic ratio is more similar to that of the first than any of the intervening ones. These fluctuations could reflect changing genetic composition of the stock perhaps due to sampling error in the bottleneck of five pairs in each generation. They also could be related to the generational sequence.

Consecutive passage of more than two generations of *Pieris protodice* through a *"vernalis"*-inducing regime, with or without diapause, probably never occurs in nature. Normally the autumnal generation, composed of intermediate and *"vernalis"* phenotypes, without diapause, is followed in spring by an all-*"vernalis"* brood which has diapaused and which produces the first of several summer generations. In the constant short-day regime, second-, third-, and fourth-generation *vernalis* were somewhat darker than first, and the fifth brood was mixed. This was true even though the incidence of non-*vernalis* phenotypes increased after the second generation; the result was a maximum phenotypic variance in the fourth. This suggests that the environmental physiology of the parent(s) may play a role in phenotypic determination of the present generation, presumably through extranuclear mechanisms. Similar effects on diapause are well-known in various insects, especially parasitic Hymenoptera (Beck, 1968). A maternal influence in "congenital" host preference is on record in *Pieris rapae* (Hovanitz, 1970).

One test carried out with Brooklyn *P. protodice* bears directly on this point. Progeny of one long-day female were reared on both long- and short-day regimes. of 25 larvae reared on 14 hours light, 22 gave light adults, 3 intermediate, 0 dark. Of 24 on 8 hours, there were 0 light, 3 intermediate, and 21 *"vernalis."* In each lot the butterflies were allowed to mate and oviposit freely, and all eggs laid in the first two days were reared on the short day. There were 56 butterflies from the short-day parents, giving 1 light: 10 intermediate: 45 dark (3 "chevronless"). Of 31 from the long-day parents there were 0, 2, and 29 respectively, with no "chevronless". These ratios are not significantly different. However, the pattern was conspicuously darker on the second-generation short-day butterflies.

Unfortunately, the broods reported here were reared on field-collected New York City *Lepidium virginicum* Linnaeus (Cruciferae); although rearing conditions were otherwise quite uniform, the possibility of effects mediated through the chemistry or condition of the host plants should be rigorously excluded before internal mechanisms are sought. However, the rearing sequence for the last experiment reported was carried out at the same time as the latter part of the five-generation experiment, and plants collected at the same time and place were not associated with similar results.

In the montane species *Pieris occidentalis* Reakirt rearing on a long day does not fully suppress the dark phenotype (Shapiro, in press). If carryover effects do influence phenotypic determination in *Pieris*, this may be a factor in maintaining the variability of *P. occidentalis* where it is bivoltine.

Abstract.–The short-day phenotype of *Pieris protodice* is subject to modification in the second and subsequent generations of continuous rearing on a short-day regime. The expression of this shift is apparently related to a carryover effect from prior generations; the frequency of the abnormal phenotype increased but subsequently decreased over the five-generation period of the experiment.–Arthur M. Shapiro, Department of Zoology, University of California, Davis, California 95616.

Descriptors: Pieris protodice; photoperiodism; phenotypic switch mechanism; carryover effect.

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Figure 1. Short-day *("vernalis")* bred male *Pieris protodice:* chevroned (wild-type) right, Chevronless left.

Table 1. Phenotypic ratios in broods of Brooklyn, N.Y. Pieris protodice reared on 8L:16D at 25°C. At the start of the experiment the stock had been reared through one long-day generation (see text). "Dark" phenotypes are "vernalis."

Generation on regime	Phenotype:	Light	Intermediate	Frequency (light + intermediate)	Dark:Chevroned	Chevronless	Frequency chevronless (dark only)
1		0	6	0.06	99	0	0.00
2		0	3	0.03	81	26	0.24
3		1	11	0.15	54	15	0.22
4		5	16	0.33	35	8	0.19
5		2	10	0.14	66	5	0.07

Table 2. Phenotypic ratios in single crosses of Pieris protodice G_3 reared on 8L:16D at 25°C.

Cross	Phenotype:	Light	Intermediate	Frequency (light + intermediate)	Dark:Chevroned	Chevronless	Frequency chevronless (dark only)
Chevroned ♂ X Chevroned ♀		5	6	0.21	35	8	0.15
Unchevroned ♂ X Unchevroned ♀		1	3	0.14	19	6	0.21